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L5: Entry 1 of 8 File: USPT Jun 15, 1999

DOCUMENT-IDENTIFIER: US 5912117 A
TITLE: Method for diagnosis of lyme disease

DEPR:
After amplification by PCR, the target polynucleotides may be detected directly by gel analysis provided the target DNA is efficiently. amplified and the primers are highly specific to the target region to be amplified. To assure PCR efficiency, glycerol and other related solvents such as dimethyl sulfoxide, can be used to increase the sensitivity of the PCR at the amplification level and to overcome problems pertaining to the sequencing of regions of DNA having strong secondary structure. These problems may include: (1) low efficiency of the PCR, due to a high frequency of templates that are not fully extended by the polymerizing agent or (2) incomplete denaturation of the duplex DNA at high temperature, due to high GC content. The use of such solvents increases the sensitivity of the assay at the level of amplification to approximately several femtograms of DNA (which is believed to correspond to a single spirochete cell). This level of sensitivity eliminates the need to detect amplified target DNA using a probe, and thereby dispenses with the requirements for radioactive probes, gel electrophoresis, Southern blotting, filter hybridization, washing and autoradiography. The concentration range for glycerol is about 5%-20% (v/v), with 10% being preferred, and the DMSO concentration range is about 3%-10% (v/v).

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Search Results -

Terms	Documents
l3 same advantag$	1

Database: US Patents Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

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Search History

Today's Date: 10/19/2000

DB Name	Query	Hit Count	Set Name
USPT	l3 same advantag$	1	L4
USPT	l2 same (DNA or oligonucleotide)	332	L3
USPT	l1 same (amplif$ or PCR)	507	L2
USPT	DMSO or (dimethyl near0 sulfoxide)	54819	L1

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L4: Entry 1 of 1 File: USPT Feb 13, 1996

DOCUMENT-IDENTIFIER: US 5491086 A
TITLE: Purified thermostable nucleic acid polymerase and DNA coding sequences from pyrodictium species

DEPR:
Higher temperatures may be required as the buffer salt concentration and/or GC composition of the nucleic acid is increased. The Pyrodictium enzymes do not become irreversibly denatured from relatively short exposures to temperatures of about 95.degree. C.-100.degree. C. The extreme thermostability of the Pyrodictium DNA polymerase enzymes provides additional advantages over previously characterized thermostable enzymes. Prior to the present invention, efficient PCR at denaturation temperatures as high as 100.degree. C. had not been demonstrated. No thermostable DNA polymerases have been described for this purpose. However, as the G/C content of a target nucleic acid increases, the temperature necessary to denature (T.sub.den), the duplex also increases. For target sequences that require a T.sub.den step of over 95.degree. C., previous protocols require that solvents are included in the PCR for partially destabilizing the duplex, thus, lowering the effective T.sub.den. Agents such as glycerol, DMSO, or formamide have been used in this manner in PCR (Korge et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89:910-914, and Wong et al., 1991, Nuc. Acids Res. 19:225 1- 2259, incorporated herein by reference). These agents, in addition to destabilizing duplex DNA will affect primer stability, can inhibit enzyme activity, and varying concentrations of DMSO or formamide decrease the thermoresistance (i.e., half-life) of thermophilic DNA polymerases. Accordingly, a significant number of optimization experiments and reaction conditions need to be evaluated when utilizing these cosolvents. In contrast, simply raising the T.sub.den to 100.degree. C. with Pot or Pab DNA polymerase in an otherwise standard PCR can facilitate complete strand separation of PCR product eliminating the need for DNA helix destabilizing agents.

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Terms	Documents
l5 same advantag$	1

Database: US Patents Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

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Today's Date: 10/19/2000

DB Name	Query	Hit Count	Set Name
USPT	l5 same advantag$	1	L7
USPT	l5 same Tm	0	L6
USPT	l1 same (duplex near0 DNA)	8	L5
USPT	l3 same advantag$	1	L4
USPT	l2 same (DNA or oligonucleotide)	332	L3
USPT	l1 same (amplif$ or PCR)	507	L2
USPT	DMSO or (dimethyl near0 sulfoxide)	54819	L1